

# Growth response of four cccessions of *Moringa oleifera* Linn shoots cultured on various basic media

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**Abstract.** *Moringa* (*Moringa oleifera* Linn) has a highly considerable nutritional value. The conventional propagation of *Moringa* has limitation. Propagation through tissue culture offers uniform transplants, and free of pests and diseases. Selection of basic medium is a critical point to find out the best medium compositions for micropropagation. The research was aimed to investigate growth response of *M. oleifera* shoots cultured on several basic media. The experimental design was factorial complete random design with two factors i.e. types of basic medium (MS, WPM, DKW, NN, and B5) in combination with accessions of *M. oleifera*: Bogor, Pekalongan, Bima and Blora. Each treatment consisted of 12 replicates. The growth variables observed were shoot height, number of shoots, number of petioles, number of roots, fresh weight, dry weight of plantlets, and plantlet performance. The growth observation was carried out every week. The results showed that type of basic medium significantly affected shoot height, number of petioles, fresh weight, dry weight, and number of shoots, but, it did not significantly affect the number of roots. The accessions significantly affected shoot height, number of petioles, number of roots, fresh weight, and dry weight, but, did not significantly affect number of shoots. The ANOVA showed an interaction between basic medium in combination with accessions on all growth variables observed. The highest shoot height (13,97 cm), number of petioles (12,33), and number of roots (8.33) were achieved on DKW basic medium on Bima and Pekalongan accessions, while the highest number of shoots (6.33) was found in NN medium.

**Keywords:** Micropropagation, Growth, Shoots, Drumstick (*M. oleifera*), Basic Media

## 1. Introduction

*Moringa oleifera* Linn is known as a plant species having high nutritional value. The World Health Organization (WHO) has socialized moringa as an alternative food to overcome malnutrition, especially for pregnant and lactating women [1]. In several countries, moringa has been widely used as a dietary supplement because it has many nutrients useful for mothers and infants in their breastfeeding phase.

All parts of *Moringa* plant have the nutritional value that can be improving health conditions [2]. The pods, flowers, and young leaves are consumed as culinary ingredients, while the roots and seeds are used as herbal medicines. The leaves and pods contained many vitamins and minerals. Several studies have been carried out on the benefits of moringa, including as an anti-hypersensitive,



antipyretic, and anti-microbial agent [3]. Moringa leaves have contain calcium, iron, protein, vitamin A, vitamin B, and vitamin C [4]. The leaves also contain higher iron than other vegetables, which about 17.2 mg/100 g [5]. Besides the leaves have vitamin C equivalent to vitamin C in seven oranges, vitamin A equivalent to vitamin A in four carrots, calcium equivalent to calcium in four glasses of milk, potassium equivalent to three bananas, and protein equivalent to two bottles of yoghurt [6].

Several studies showed that the moringa plant contains saponins, flavonoids, and other secondary metabolite compounds, including arginine and glutamic acid, which play a role in suppressing the effect of hypoglycemic occurrence in experimental animals [7]. This plant is believed to be able to cure various diseases, including diabetes mellitus, hepatoprotective, immunomodulatory, and anti-inflammatory [8] [9].

Conventional propagation of Moringa is generally by seeds or vegetative propagation by cuttings. Conventional propagation is susceptible to some diseases such as fungi, bacteria, and viruses. A limited number of explants for cuttings and seed viability are an obstacle to scaling up seedling production. Propagation through plant tissue culture technique can be applied to produce uniform seeds, transplants free from pests and diseases, stable, and can be produced in a relatively short time. [10].

Basic medium is one of the critical factors in tissue culture propagation. The optimal media composition for plant growth varied according to the plant type [11]. The basic media composition was formulated to optimize plant growth and development; it generally consists of macro and microelements, amino acids, vitamins, organic materials, and carbon sources [12].

MS (Murashige and Skoog) is a medium commonly used in plant tissue culture. It contains complete nutrients including macronutrient elements: Ca, Mg, N, K, S, and P, micronutrient elements: B, Cu, Co, Mo, Mn, Na, and Zn, vitamins, elements of organic matter, and sources carbon. This media contains nitrogen and potassium macronutrients which are high enough to induce plant regeneration [13]. WPM medium (Woody Plant Medium) was first developed in 1981 by Lloyd and McCoen, intended for tissue culture of woody plants because it contains high sulphates with low total ionic levels. There are macro elements in this medium, including 900 mg of  $K_2SO_4$  and supplemented with  $NH_4NO_3$ ,  $MgSO_4$ ,  $Ca(NO_3)_2$ , and vitamins. Besides macro elements, there are also microelements:  $Na_2EDTA$ ,  $FeSO_4$ ,  $H_3BO_3$ ,  $MnSO_4$ ,  $Na_2MoO_4$ , and also  $ZnSO_4$  [14].

DKW (Driver and Kuniyaki Walnut) is initially used for shoot plant propagation and callus development on Walnut plant. This medium has a higher Calcium element compared to MS medium, which is useful for helping plant explant growth. Apart from Calcium, other macroelements include magnesium, potassium, sodium, chloride, sulfate, and micronutrients: Mn, B, Cu, Ni, and Zn. The highest micronutrient content is manganese (198 $\mu$ M), and the lowest micronutrient content is nickel (0.02 $\mu$ M) [15].

At first, NN (Nitsch and Nitsch) was a medium used to initiate haploid culture (pollen culture) on tobacco plants. However, currently, NN medium is commonly used for *in vitro* plant propagation of many plant species. NN medium contains high macronutrients elements:  $CaCl_2$ ,  $KH_2PO_4$ ,  $KNO_3$ ,  $MgSO_4$ ,  $NH_4NO_3$ ,  $NO_3^-$  and  $K^+$ . Whereas micronutrients:  $CuSO_4 \cdot 5H_2O$ ,  $FeNaEDTA$ ,  $H_3BO_3$ ,  $MnSO_4 \cdot H_2O$ ,  $Na_2MoO_4 \cdot 2H_2O$ , and  $ZnSO_4 \cdot 7H_2O$ . Nitsch & Nitsch medium was optimal for multiplication of anthurium plants, when it was combined with 1 mg/l BAP resulted in a high number of shoots and length of anthurium shoots [11].

Medium B5 (Gamborg) contains macro and micronutrients, vitamins, calcium chloride, and potassium iodide. The micronutrients contained in B5 media:  $MnSO_4 \cdot H_2O$ ,  $H_3BO_3$ ,  $ZnSO_4 \cdot 7H_2O$ ,  $Na_2MoO_4 \cdot 2H_2O$ , and  $CuSO_4 \cdot 5H_2O$ . Meanwhile, vitamins include nicotine acid, thiamin HCL, Pyridoxine HCL, and myoinositol [14]. Medium B5 supplemented with 2 mg/l BAP produces an excellent response for plant shoot growth of banana on *in vitro* culture [16].

In addition to the basic media, several factors which influence tissue culture are age of plant, age of explants, type of explants, plant growth regulators, and methods that are commonly used in tissue culture technique [17]. This researched aimed to determine the effect of different basic media on the *M. oleifera* growth on *in vitro* culture and to determine the optimum basic media for shoot propagation.

## 2. Materials and Methods

### 2.1 Materials

Materials used in this study were plantlets of 4 accessions of *M. oleifera* originally from Bogor, Pekalongan, Bima, and Blora grown on DKW media supplemented with 1 mg/l BAP six weeks after culture. In this experiment, internodes of *M. oleifera* at 1.5-2.0 cm long, having 2-3 leaves were cultured according to the medium treatment tested. The culture media used were five basic media namely MS, WPM, DKW, NN, and B5 Gamborg media supplemented with 30 g/l of sucrose. The media were solidified with 3 g/l of Gelzan (TM Caissonlabs). Medium pH was adjusted to 5.8 before sterilization using autoclave at 15 psi at 121°C for 20 min. Cultures were incubated at  $26 \pm 2^\circ\text{C}$ , with continuous photoperiod with a light intensity of 500-900 lux.

### 2.2 Experimental Design and Data Analysis

The experimental design used was factorial Completely Randomized Design (CRD) with two factors. The first factor was five basic media: MS, WPM, DKW, NN, and B5 Gamborg, while the second factor was four accessions of *M. oleifera* collected from Bogor, Pekalongan, Bima, and Blora. Quantitative data were analyzed using Analysis of Variance (ANOVA) to determine the effect between treatments. The significantly different variables were tested using Duncan's Multiple Range Test (DMRT) at  $\alpha$  1 and 5% levels using DSASTAT V.1.1 (open-source software). Compositions of the basic media are presented in Table 1.

Each treatment consisted of 4 bottles, each bottle containing three explants as replicates, therefore, the total number of experimental units was 240. The variables observed were shoot height, number of shoots, number of petioles, number of roots, fresh weight, dry weight of plantlets, and plantlet performance. Observation of shoot height, number of shoots, number of petioles, and number of roots was carried out once a week from 0 to 6 weeks after culture. Observation of fresh weight, dry weight of plantlets, and plantlet performance were carried out at six weeks of age.

Table 2. MS, WPM, DKW, NN dan B5 media Composition

Composition	MS <sup>1</sup> (mg/l)	WPM <sup>2</sup> (mg/l)	DKW <sup>3</sup> (mg/l)	NN <sup>4</sup> (mg/l)	B5 <sup>5</sup> (mg/l)
NH <sub>4</sub> NO <sub>3</sub>	1650	400	17.600	720	0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	0	0	0	134
C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub> S	0	0	0	0.05	0
H <sub>3</sub> BO <sub>3</sub> (Boric Acid)	6.2	6.2	0.078	10	3
CaCl <sub>2</sub>	332.2	72.47	1.010	166	113.24
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.025	0	0	0	0.025
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.025	0	0	0.03	0.025
C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> .2H <sub>2</sub> O	0	0	0	37.26	0
C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> NaFeO <sub>8</sub> .3H <sub>2</sub> O (EDTA)	37.26	37.3	120	0	36.7
C <sub>19</sub> H <sub>19</sub> N <sub>7</sub> O <sub>6</sub> (Folic Acid)	0	0	0	0.5	0
FeSO <sub>4</sub> .7H <sub>2</sub> O	27.8	27.85	0	0	0
C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub> (Glycine)	0	2	0.0266	2	0
MgSO <sub>4</sub>	180.7	180.7	3.000	90.37	122,09
MnSO <sub>4</sub> .H <sub>2</sub> O	16.9	22.3	0.200	18.9	10
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.25	0.25	0.002	0.25	0.25

C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> (Myo – Inositol)	100	100	0.5600	100	100
C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> (Nicotinic Acid)	0.5	0.5	0	5	1
KI	0.83	0	0	0	0.75
KNO <sub>3</sub>	1900	0	0	950	2500
KH <sub>2</sub> PO <sub>4</sub>	170	0	1.950	68	0
C <sub>8</sub> H <sub>11</sub> NO <sub>3</sub> .HCl (Pyridoxine)	0.5	0.5	0	0.5	1
NaH <sub>2</sub> PO <sub>4</sub> . H <sub>2</sub> O	0	0	0	0	150.08
C <sub>12</sub> H <sub>17</sub> CIN <sub>4</sub> OS.HCl (Thiamine)	100	1	59	0.5	10
ZnSO <sub>4</sub> . 7H <sub>2</sub> O	8.6	8.6	0.072	10	2
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	0	556	8.300	0	0
CuSO <sub>4</sub>	0	0.16	0	0	0
K <sub>2</sub> SO <sub>4</sub>	0	990	8.950	0	0

Noted: <sup>1</sup>Murashige & Skoog (1962) [18]; <sup>2</sup>McCown & Lloyd (1981) [19]; <sup>3</sup>Driver & Kuniyuki (1984) [20]; <sup>4</sup>Nitsch & Nitsch (1969) [21]; <sup>5</sup>Gamborg *et al.*, (1968) [22]

### 3. Results and Discussion

The growth of four the accession of *M. oleifera* shoot cultured on the five basic medium treatment MS, WPM, DKW, NN, and Gamborg is shown in Figure 1. In Bogor accession, the growth shoot height began at 2 weeks after culture. In MS and DKW medium, optimal shoot height growth was at 3-6 weeks. On WPM, NN, and B5 media, the growth of *M. oleifera* cultures was slower than in MS and DKW media (Figure 1A). In Pekalongan accession with DKW-basic media, shoot height showed a significant increase at 2-6 weeks after culture. Whereas in MS, WPM, NN, and Gamborg, shoots' height has a slow growth response (Figure 1B).

In Bima's accession, the height of shoot growth was higher than that in others, the optimal shoots growth of *M. oleifera* was on DKW medium. The growth of culture began 1 week after culture. The increase of shoot height continued and showed a significantly increase until 6 weeks after culture. This medium produced the highest shoot height of *M. oleifera* compared to other treatments. On MS, NN, and B5 has the same growth performance, while on WPM medium, it has medium shoot height growth (Figure 1C). In line with these results, in Blora accession shoot height growth was also optimal in the DKW media, which increased at 2-6 weeks after culture. In MS, WPM, NN, and B5 media shoot height were not significantly different (Figure 1D). DKW media has more suitable for growth than other media; the nutritional content in DKW media has higher phosphorus elements (Table 1) that are beneficial for plants and play an important role in the formation of albumin and accelerate cell division for stem and leaves. Phosphorus nutrients also function to strengthen stems, improve plant quality, shoot development, and increase plant resistance to disease [23] [24].

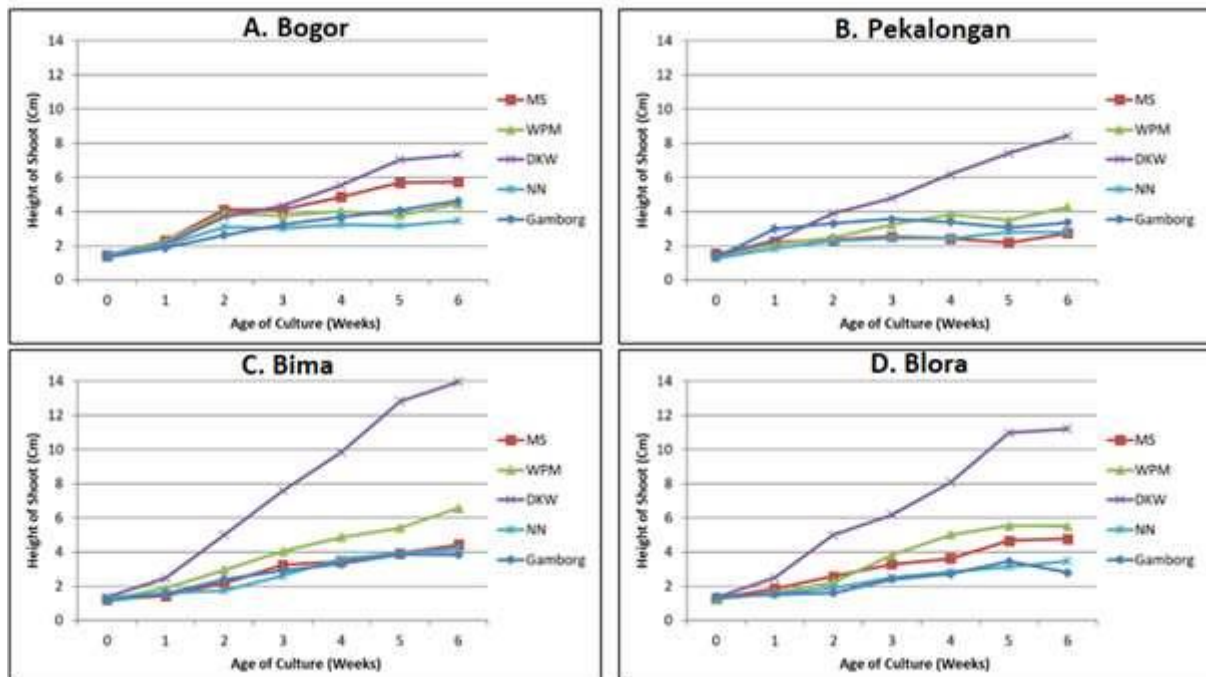


Figure 1. Growth of shoot height of four accessions of *M. oleifera*, Bogor (A), Pekalongan (B), Bima (C), and Blora (D) cultured for 0-6 weeks on five basic media MS, WPM, DKW, NN and Gamborg.

The increasing number of shoots of four accessions of *M. oleifera* in MS, WPM, DKW, NN, and Gamborg media is shown in Figure 2. In Bogor accession, the increasing number of shoots began at 2 and 3 weeks after culture. The highest number of shoots was found in WPM medium, which had optimal growth at 3-6 weeks after culture. The increase in number of shoots on DKW and MS media relatively the same at 2-6 weeks on culture period, while on the NN and B5 media, the increasing number of shoots at six weeks was not significantly different (Figure 2A). In Pekalongan accession, the number of shoots of *M. oleifera* was quite varied on different media. An increase in the number of shoots began two weeks after culture. The optimal number of shoots shown at 3-6 weeks after culture. In the last observation at six weeks after culture, the highest number of shoots was found in WPM media, followed by MS, DKW, NN, and B5 (Figure 2B).

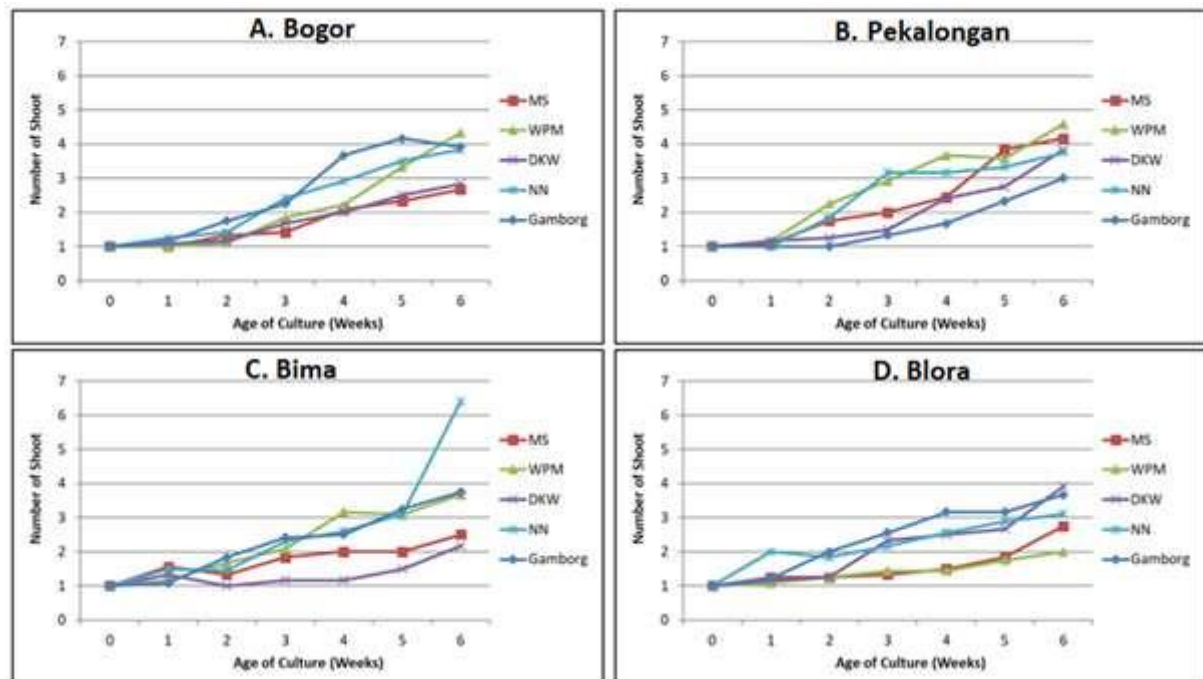


Figure 2. Growth of the number of shoots of four accessions of *M. oleifera*, Bogor (A), Pekalongan (B), Bima (C), and Blora (D) cultured for 0-6 weeks on five basic media MS, WPM, DKW, NN and B5.

In Bima accession, the increasing number of shoots on DKW medium was lower than in MS, WPM, NN, and B5 media. The highest number of shoots was found in the NN medium with optimal growth at 2-6 weeks after culture (Figure 2C). In Blora accession, high growth in shoot numbers was found on DKW and B5 media, where the number of shoots continued to increase from 3 to 6 weeks after culture. In the WPM medium, the number of shoots was the lowest than others at 2-6 weeks after culture (Figure 2D).

Figure 3 shows the increasing number of petioles in *M. oleifera* cultured on MS, WPM, DKW, NN, and B5 media at 0 to 6 weeks after culture. In Bogor accession, the number of petioles increased two weeks after culture. In MS media, the optimal number of petioles was increased at 4-6 weeks after culture. At the age of 6 weeks after culture, the number of petioles in all basic media treatments was not significantly different (Figure 3A). In Pekalongan accession, the number of petioles varied in each basic media treatment. In the WPM, DKW, NN, and B5 media, the number of petioles began to increase at two weeks after culture, while on MS media, the number of petioles increased at four weeks after culture. At six weeks after culture, the highest number of petioles was found in DKW and WPM media, then followed by NN, MS media, and the lowest was B5 media (Figure 3B).

In Bima accession, the number of petioles increased at two weeks after culture in all basic media. In WPM medium, the optimal increasing number of petioles occurred at 2-6 weeks after culture. In this medium, the number of petioles was higher than that in DKW, NN, B5, and MS media (Figure 3C). In Blora accession, the highest increase in the number of petioles was found in the DKW medium, where the increasing number of petioles began one week after culture. The number of petioles continued to increase until six weeks after culture. The number of petioles on this medium was higher at six weeks after culture than in NN, MS, WPM, and B5 media (Figure 3D).

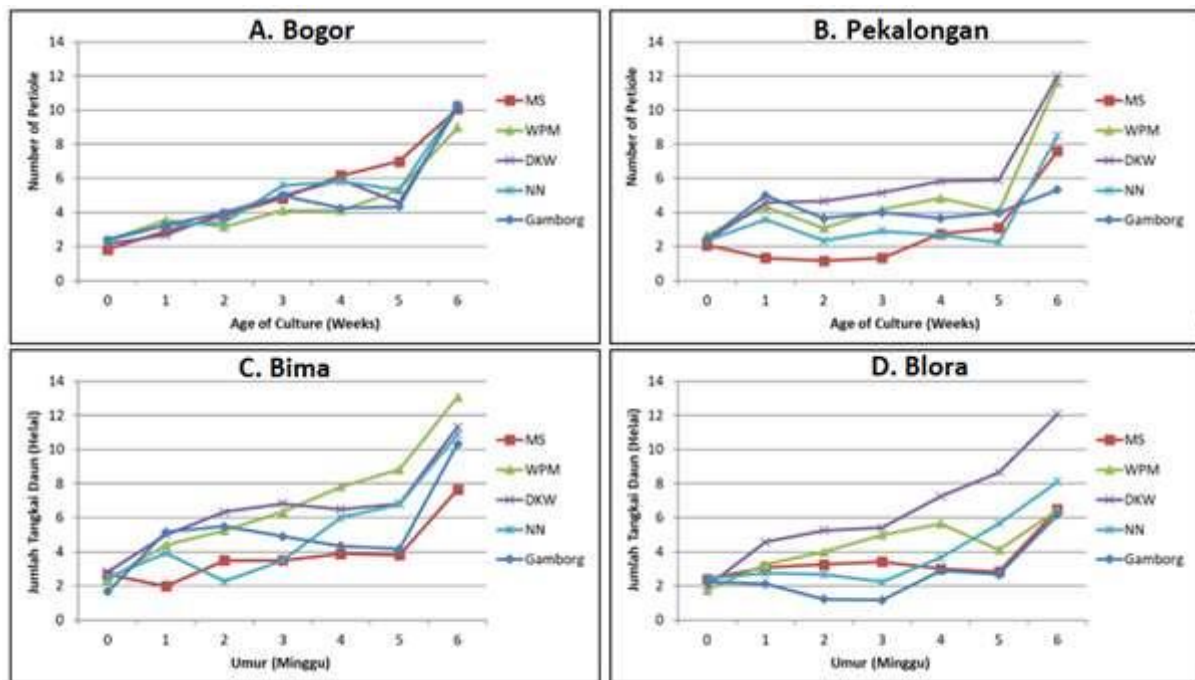


Figure 3. Growth of the number of petioles of four accessions of *M. oleifera*, Bogor (A), Pekalongan (B), Bima (C), and Blora (D) cultured for 0-6 weeks on five basic media MS, WPM, DKW, NN and B5.

The number of roots in four accessions of *M. oleifera* cultured on MS, WPM, DKW, NN, and B5 media shown in Figure 4. In Bogor accessions, roots began to form three weeks after culture. The average number of roots on MS, DKW, and B5 media at 2-6 weeks was not significantly different. A small number of roots was found in the NN and WPM media (Figure 4A). In Pekalongan accession, the highest number of roots was found in B5 media, where the increasing number of roots began at four weeks and continued to increase until six weeks after culture. In the NN, DKW, WPM, and MS media, only a small number of roots was formed (Figure 4B).

In Bima Accession, roots started to form two weeks after culture. In DKW medium, the optimal number of roots increased from 3 to 6 weeks. In this medium, the number of roots was higher than in MS, WPM, NN, and B5 media. In MS and WPM media, roots were formed at three weeks, the optimal number of roots increased four weeks after culture. In NN and B5 media, only a small number of roots was formed (Figure 4C). In Blora accession cultured in MS, WPM, and NN media, the number of roots increased two weeks after culture, while in DKW and B5 media, the formation of roots occurred three weeks after culture. In MS medium, the optimal number of roots increased from 3 to 6 weeks after culture, while in the NN, DKW B5, and WPM media, the number of roots formed was lower than in MS medium (Figure 4D). The content of vitamin B1 (thiamine) on MS medium was higher than that in other media. Thiamine affects root growth where the vitamin works as a coenzyme that can stimulate auxin synthesis. The synthesis of this auxin in plants will trigger cell enlargement and cell elongation in the meristem area. This hormone accelerates plant development, especially in root growth [24].



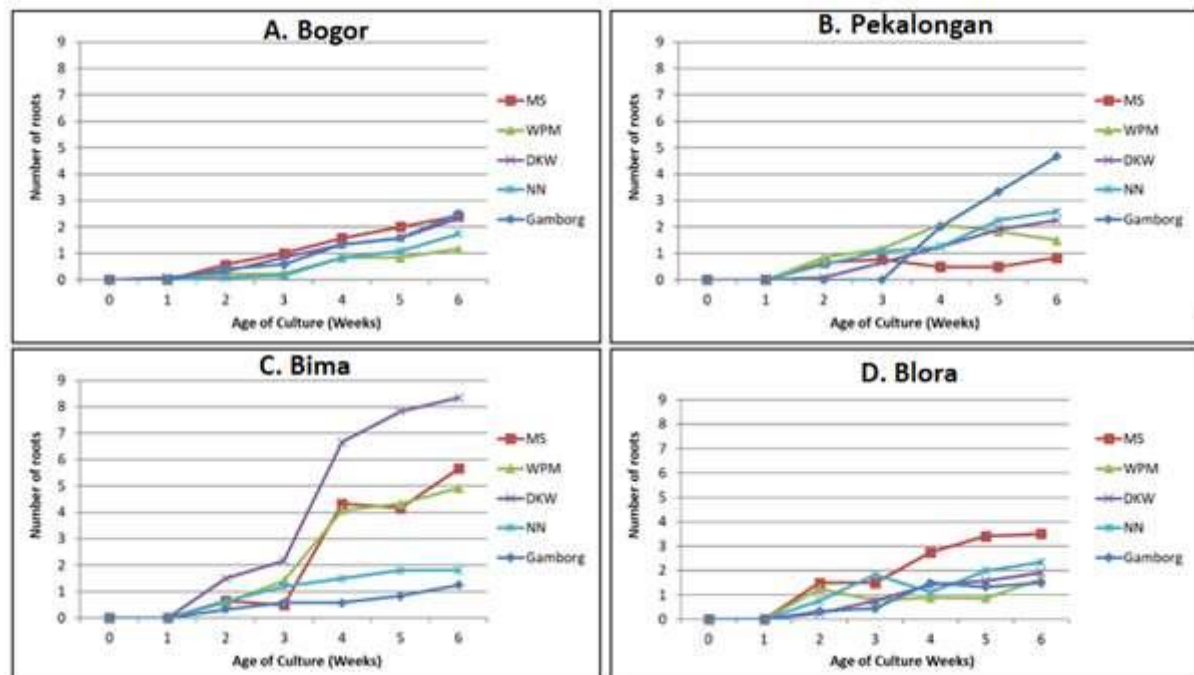


Figure 4. Growth of the number of shoots of four accessions of *M. oleifera*, Bogor (A), Pekalongan (B), Bima (C), and Blora (D) cultured for 0-6 weeks on five basic media MS, WPM, DKW, NN and B5.

Analysis of variance of shoot height, number of shoots, number of petioles, number of roots, fresh weight, and dry weight of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora cultured on MS, WPM, DKW, NN, and B5 media is shown in Table 2.

Table 2. Analysis of variance (ANOVA) on shoot height, number of shoots, number of petioles, number of roots, fresh weight, and dry weight of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora cultured on MS, WPM, DKW, NN and B5 media weeks after culture

No	Variables	F Value & Significance			CV (%)
		Basic Media	Accession	Basic Media Vs Accession	
1.	Shoot height	89,83**	11,93**	5,87**	26,81
2.	Number of Shoots	2,78*	1,24 <sup>ns</sup>	3,02**	42,60
3.	Number of Petioles	10,48**	8,74**	4,15**	23,44
4.	Number of Roots	1,59 <sup>ns</sup>	6,14**	3,24**	87,67
5.	Fresh weight	17,37**	10,53**	6,97**	40,79
6.	Dry Weight	13,17**	9,31**	5,19**	45,54

Noted \* : significance at  $\alpha$ : 5%; \*\*: very significance at  $\alpha$  1%; ns : not significance

In the basic media treatment, there was a highly significant effect on the variables of shoot height, number of petioles, fresh weight, dry weight, and a significant effect on the variable number of shoots, but not significantly different on the number of roots. In comparison, the type of accession significantly affected the variables of shoot height, number of petioles, number of roots, fresh weight, and dry weight but not significantly on the number of shoots. The ANOVA table also showed an interaction between basic media and accession on all variables observed (Table 2).



The average shoot height, number of shoots, number of petioles, and number of roots of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora *M. oleifera* cultured on MS, WPM, DKW, NN and B5 media 6 weeks after culture were shown in Table 3. The highest shoot was found in Bima accession cultured on DKW medium which was significantly different from other media. The lowest shoot was found in MS media of Pekalongan accession, NN media of Bogor accession, and B5 of Pekalongan and Blora accessions (Table 3).

Table 3. The average value of shoot height, number of shoots, number of petioles, and number of roots of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora *M. oleifera* cultured on MS, WPM, DKW, NN and B5 media 6 weeks after culture

Basic Medium	Accession	Shoot height (cm)	Number of Shoots	Number of Petioles	Number of Roots
MS	Bogor	5,73 ± 0,50 <sup>def</sup>	2,67 ± 0,33 <sup>bcd</sup>	10,17 ± 0,31 <sup>abc</sup>	2,33 ± 0,33 <sup>cde</sup>
	Pekalongan	2,75 ± 0,16 <sup>g</sup>	4,17 ± 0,95 <sup>bc</sup>	7,60 ± 1,05 <sup>cde</sup>	0,83 ± 0,54 <sup>e</sup>
	Bima	4,43 ± 1,00 <sup>fg</sup>	2,00 ± 0,45 <sup>d</sup>	7,67 ± 1,41 <sup>cde</sup>	5,67 ± 2,42 <sup>ab</sup>
	Blora	4,70 ± 0,45 <sup>fg</sup>	2,83 ± 0,31 <sup>bcd</sup>	6,67 ± 0,92 <sup>de</sup>	3,50 ± 0,43 <sup>bcdde</sup>
WPM	Bogor	4,45 ± 0,53 <sup>fg</sup>	4,33 ± 0,61 <sup>b</sup>	9,00 ± 0,68 <sup>bcd</sup>	1,17 ± 0,40 <sup>e</sup>
	Pekalongan	4,48 ± 0,80 <sup>fg</sup>	4,50 ± 0,88 <sup>b</sup>	11,33 ± 0,95 <sup>ab</sup>	2,17 ± 0,79 <sup>cde</sup>
	Bima	6,65 ± 0,79 <sup>de</sup>	3,67 ± 0,80 <sup>bcd</sup>	<b>13,17 ± 1,25<sup>a</sup></b>	4,83 ± 1,30 <sup>bc</sup>
	Blora	5,43 ± 0,63 <sup>ef</sup>	2,00 ± 0,37 <sup>d</sup>	6,33 ± 0,67 <sup>de</sup>	1,67 ± 0,33 <sup>cde</sup>
DKW	Bogor	7,28 ± 0,74 <sup>cd</sup>	2,83 ± 0,60 <sup>bcd</sup>	10,33 ± 1,54 <sup>abc</sup>	2,33 ± 0,21 <sup>cde</sup>
	Pekalongan	8,45 ± 0,84 <sup>c</sup>	3,83 ± 0,40 <sup>bcd</sup>	<b>12,00 ± 1,00<sup>a</sup></b>	2,33 ± 0,21 <sup>cde</sup>
	Bima	<b>13,97 ± 0,76<sup>a</sup></b>	2,17 ± 0,40 <sup>cd</sup>	11,33 ± 0,56 <sup>ab</sup>	<b>8,33 ± 2,35<sup>a</sup></b>
	Blora	11,20 ± 0,80 <sup>b</sup>	3,83 ± 0,83 <sup>bcd</sup>	<b>12,33 ± 0,67<sup>a</sup></b>	1,83 ± 0,31 <sup>cde</sup>
NN	Bogor	3,37 ± 0,12 <sup>g</sup>	3,83 ± 0,40 <sup>bcd</sup>	10,50 ± 0,56 <sup>abc</sup>	1,83 ± 0,54 <sup>cde</sup>
	Pekalongan	2,88 ± 0,27 <sup>g</sup>	3,67 ± 0,33 <sup>bcd</sup>	8,50 ± 0,67 <sup>bcd</sup>	2,50 ± 0,67 <sup>cde</sup>
	Bima	3,97 ± 0,46 <sup>fg</sup>	<b>6,33 ± 0,88<sup>a</sup></b>	10,50 ± 0,56 <sup>abc</sup>	1,83 ± 0,31 <sup>cde</sup>
	Blora	3,43 ± 0,54 <sup>g</sup>	3,17 ± 0,70 <sup>bcd</sup>	8,17 ± 1,35 <sup>cde</sup>	2,33 ± 0,61 <sup>cde</sup>
B5	Bogor	4,65 ± 0,57 <sup>fg</sup>	3,83 ± 0,65 <sup>bcd</sup>	10,33 ± 0,56 <sup>abc</sup>	2,50 ± 0,34 <sup>cde</sup>
	Pekalongan	3,37 ± 0,24 <sup>g</sup>	3,00 ± 0,58 <sup>bcd</sup>	5,33 ± 0,42 <sup>e</sup>	4,67 ± 1,84 <sup>bcd</sup>
	Bima	3,82 ± 0,24 <sup>fg</sup>	3,67 ± 0,56 <sup>bcd</sup>	10,33 ± 0,67 <sup>abc</sup>	1,33 ± 0,33 <sup>de</sup>
	Blora	2,80 ± 0,17 <sup>g</sup>	3,67 ± 0,42 <sup>bcd</sup>	6,17 ± 0,87 <sup>de</sup>	1,50 ± 0,43 <sup>cde</sup>

Note: The numbers followed by the same letter in the same column are not significantly different based on Duncan's multiple range test at  $\alpha = 5\%$

The highest number of shoots was found in NN medium of Bima accession, significantly different from others, while the low number of shoots was found in MS medium of Bima and WPM media of Blora accession (Table 3). NN medium has sufficient nutritional levels with high nitrogen content, which is quite essential for plant growth. Nitrogen can stimulate plant growth, both for leaf and stem growth. Nitrogen is a constituent component of all proteins and nucleic acids, which are crucial for the plant itself. This can also be caused by the low mineral content in the NN medium to stimulate shoots growth of Bima accession. The low mineral content can provide an excellent response to several types of plants such as tobacco, tomato, and potato [14][16].

The large number of petioles found in the WPM medium of Bima accession, and DKW media of Pekalongan and Blora accessions. The number of petioles tended to be lower on B5 medium, especially in Pekalongan and Blora accessions (Table 3). WPM and DKW media may have calcium, nitrogen, and potassium suitable for *M. oleifera* growth. Calcium plays an essential role in stimulating the growth of petiole, leaves, and roots and can inhibit cell aging. Nitrogen in plants functions as the formation of green color in leaves and plays a vital role in the photosynthesis process. At the same time, potassium in plants increases the photosynthesis process [23].

The highest number of roots formed at six weeks after culture was found in DKW medium of Blora accession, while the least number of roots was found in MS medium of Pekalongan accession and WPM medium of Bogor accession (Table 3). DKW medium is the best medium that can be used for root growth. This is influenced by the phosphorus and sulfur content which can trigger root growth. The content of phosphorus plays a role in the protection of the plant body, also a trigger for root growth. In comparison, sulfur also plays a role in root development which can prolong the growth of the roots. Apart from being caused by the number of elements present in the media, root growth is also influenced by the vitamin composition of a medium [25].

The mean value of the fresh weight and dry weight of *M. oleifera* shoots culture at six weeks after culture can be seen in Table 4. The highest fresh weight and dry weight were found in DKW medium of Blora accession, while the lowest raw and dry weight values were found in MS medium of Bogor accession (Table 4).

Table 4. Fresh weight and dry weight of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora *M. oleifera* shoots culture on MS, WPM, DKW, NN and B5 media 6 weeks after culture

Basic Medium	Accession	Fresh weight (mg)	Dry Weight (mg)
MS	Bogor	149,37 ± 20,17 <sup>g</sup>	11,13 ± 1,58 <sup>f</sup>
	Pekalongan	265,67 ± 53,50 <sup>fg</sup>	23,50 ± 4,19 <sup>ef</sup>
	Bima	306,93 ± 39,99 <sup>efg</sup>	25,97 ± 3,46 <sup>def</sup>
	Blora	257,83 ± 11,25 <sup>fg</sup>	26,70 ± 0,97 <sup>def</sup>
WPM	Bogor	463,07 ± 38,04 <sup>cdef</sup>	39,37 ± 2,00 <sup>cde</sup>
	Pekalongan	715,43 ± 151,23 <sup>b</sup>	60,73 ± 15,03 <sup>bc</sup>
	Bima	457,83 ± 54,64 <sup>cdef</sup>	41,27 ± 6,17 <sup>cde</sup>
	Blora	664,50 ± 7,01 <sup>bc</sup>	64,37 ± 0,77 <sup>b</sup>
DKW	Bogor	537,40 ± 90,66 <sup>bcde</sup>	36,83 ± 7,36 <sup>de</sup>
	Pekalongan	357,40 ± 45,69 <sup>defg</sup>	32,07 ± 3,82 <sup>def</sup>
	Bima	337,70 ± 13,53 <sup>defg</sup>	28,30 ± 2,72 <sup>def</sup>
	Blora	<b>1156,80 ± 105,28<sup>a</sup></b>	<b>97,37 ± 8,34<sup>a</sup></b>
NN	Bogor	572,77 ± 92,19 <sup>bcd</sup>	48,13 ± 7,77 <sup>bcd</sup>
	Pekalongan	275,90 ± 62,49 <sup>fg</sup>	25,40 ± 5,32 <sup>def</sup>
	Bima	239,67 ± 102,84 <sup>fg</sup>	28,07 ± 13,38 <sup>def</sup>
	Blora	416,10 ± 45,18 <sup>def</sup>	33,53 ± 3,29 <sup>def</sup>
B5	Bogor	365,73 ± 79,96 <sup>defg</sup>	25,73 ± 6,71 <sup>def</sup>
	Pekalongan	462,67 ± 58,14 <sup>cdef</sup>	39,47 ± 3,22 <sup>cde</sup>
	Bima	268,43 ± 74,77 <sup>fg</sup>	24,17 ± 9,28 <sup>ef</sup>
	Blora	375,13 ± 96,54 <sup>defg</sup>	31,70 ± 9,42 <sup>def</sup>

Note: The numbers followed by the same letter in the same column are not significantly different based on Duncan's multiple range test at  $\alpha = 5\%$

The performance of four accessions of *M. oleifera* Bogor, Pekalongan, Bima, and Blora cultured on MS, WPM, DKW, NN and B5 basic media is shown in Figure 5. In Bogor accession, shoot performance on DKW media was more robust than in MS, NN and B5 media. Leaves color was greener with vigorous stems and petioles. Callusing was formed at the WPM, DKW and NN media, while on MS and B5 media there was no callus formed at the base of the stems (Figure 5A).

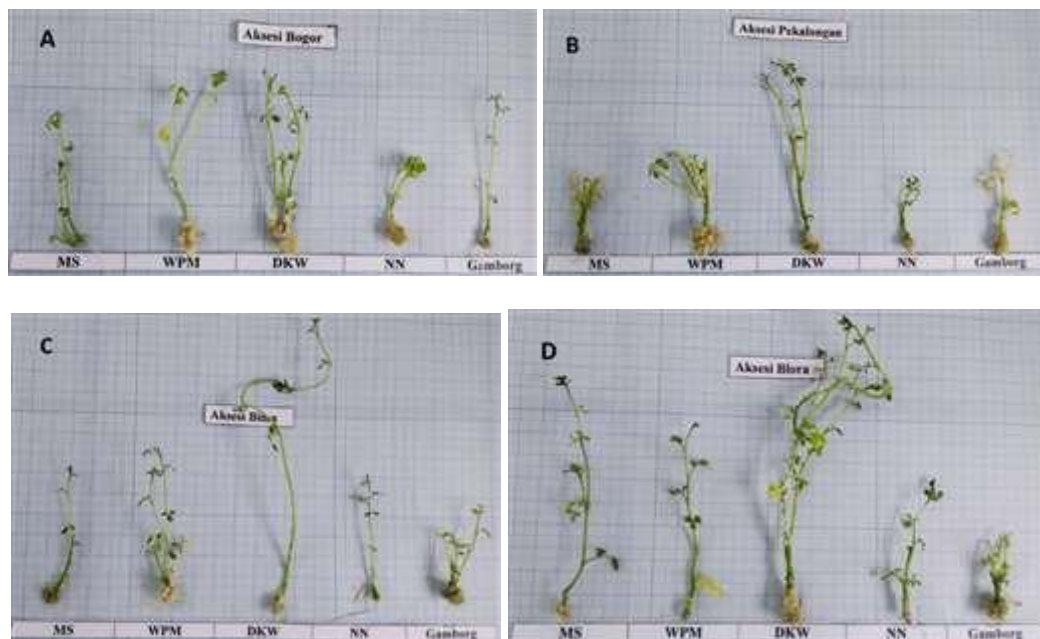


Figure 5. Culture performance of four accessions of *M. oleifera* Bogor (A), Pekalongan (B), Bima (C), and Blora (D) *M. oleifera* shoots on MS, WPM, DKW, NN, and B5 (Gamborg) media 6 weeks after culture.

In *M. oleifera* Pekalongan accession, the performance of the shoot's culture on DKW medium was much better compared to that in other media. On MS and B5 media, some parts of the stems and leaves wilted and yellowed, while on NN media the shoots did not show significant growth. At the base of stems of the culture, calli were formed in all basic media (Figure 5B). Shoots of Bima accession grown on DKW media looked vigorous with long stems and broad leaves. On MS, WPM, NN and B5 media, the shoots were stunted and had only a small number of leaves. On WPM media, DKW and B5 the bud of the stem enlarged and formed a callus (Figure 5C). In Blora accession, it can be seen that the shoot performance on DKW media is more dominant than MS, WPM, NN and B5 media which can be seen from the appearance of the vigorous stems and has many numbers of leaves. The stunted shoot performance was found in B5 media. On the DKW medium, NN and B5 at the bud of the stem formed a callus (Figure 5D). In general, DKW medium was the most suitable for the growth of *M. oleifera* shoot culture. This medium was also giving better to some other plant species such as stevia, olive, and purwoceng [23].

#### 4. Conclusion

Type of basic medium significantly affected shoot height, number of petioles, fresh weight, dry weight, and number of shoots of *M. oleifera*, but, it did not significantly affect the number of roots. The accession factor significantly affected shoot height, number of petioles, number of roots, fresh weight, and dry weight, but, did not significantly affect the number of shoots. The ANOVA showed an interaction between basic medium in combination with accessions on all growth variables observed. The highest shoot height, number of petioles, and number of roots were achieved on DKW basic medium, while the highest number of shoots was found in NN basic medium. In conclusion that DKW medium was the best for shoot growth of *M. oleifera* culture.

## Acknowledgement

The author would like to thank Aji Permana, an student from Brawijaya University, for his assistance in this research.

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